FOOTPRINT GLANDS IN *AMBLYOPONE AUSTRALIS* (FORMICIDAE, PONERINAE)

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INTRODUCTION

Social communication in ants is based primarily on chemical signals (for recent reviews see Morgan 1984; Hölldobler and Wilson, 1990). Semiochemicals (or pheromones) are produced in exocrine glands, and when discharged these substances elicit in nestmates specific behavioral patterns, such as alarm reactions and recruitment responses. In recent years the study of chemical communication has received a new impetus by the discovery of many hitherto unknown exocrine glands (see Hölldobler and Engel, 1978, 1982; Jessen et al. 1979; Hölldobler 1982; Billen 1986). In the present paper we report the discovery of special exocrine glands in the distal tarsomere of the hindlegs of *Amblyopone australis* workers. The morphology of the glands and behavioral observations suggest that foragers of *Amblyopone* mark their trails by setting "footprints" with secretions from these tarsal glands.

MATERIALS AND METHODS

Several colonies of *Amblyopone australis* were collected near Canberra (Australia) and workers were fixed in Carnoy's fixative and stored in 80% ethanol. After clearing in toluene, the legs of workers were embedded in an ultra-low viscosity epoxy medium (VCD/HXSA) as described by Mascorro et al. (1976) and Oliveira et al. (1983). Blocks were serially sectioned at 2 μ m using glass knives and a model MT2-C ultramicrotome (Research Manufacturing Company, Tucson, Arizona). Sections were attached to albumized

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Figure 1. Worker of *Amblyopone australis* returning from a food source to the nest. Its hindlegs are more tightly held to the body and with each step the distal tarsomeres of the hindlegs (arrow) are more closely pressed to the ground.

Figure 2. A recruiting *A. australis* worker moving from the nest to the food source. It exhibits the same locomotory behavior illustrated in Figure 1. It is often loosely followed by one of several nestmates.

Figure 3. Sagittal section through distal tarsomeres of the front leg of *A. australis* worker showing the arolium gland (AG) with its reservoir (AR) which directly connects with the arolium (A).

Figure 4. Close up view of a section through the arolium gland (AG) of the front leg of an *A. australis* worker. AR: reservoir of arolium gland; A: arolium.

Figure 5. Sagittal section through the fifth tarsomere of the hindleg of an *A. australis* worker, showing the massively developed footprint gland (FG) and the arolium gland (AG); A: arolium.

Figure 6. Close-up view of a section of the foot print gland with the cuticle of the fifth tarsomere of the hindleg of an *A. australis* worker; CC: capillary channels in cuticle.

glass slides and plastic was removed prior to staining with toluidine blue/basic fuchsin following Burns and Bretschneider (1981) with slight modifications.

RESULTS

An ongoing experimental analysis of the communication system of *Amblyopone australis* and *A. longidens* has revealed that these phylogenetically primitive species employ a rather complex combination of vibrational and chemical signals during recruitment to food sources (Hölldobler and Markl, unpublished). Recruiting ants conduct inside the nest a vigorous body shaking display that propagates general alert and stimulation among nestmates and chemical signals deposited along the trail of the recruiting ant give directional information about the location of the newly discovered food source. Behavioral tests clearly demonstrate that the recruiting forager as well as the recruits move along chemically marked trails. The trail following is not very precise, but in laboratory experiments a significant majority of recruits departed the nest in the direction from which the recruiting ant had returned.

Hölldobler and Markl (unpublished) also observed that the recruiting ant, when moving from the food source to the nest, changed its locomotory behavior in a subtle, but nevertheless noticeable way. It moved more slowly, holding its hind legs more tightly to the body and with each step pressed the distal tarsomeres of the hind legs more closely to the ground (Fig. 1). The ant exhibited the same behavior when leaving the nest again to return to the food source. In the latter situation it was often accompanied by a group of one or several nestmates (Fig. 2). These and other observations as well as pilot tests with extracts from hind legs suggest that *Amblyopone* workers mark trails with footprint secretions. In the following we present our histological investigations of exocrine glands in the legs of workers of *A. australis*.

We found two types of tarsal glands in *Amblyopone* workers. One type is located between the fifth tarsal segment and the pretarsal claws at the arolium (Fig. 3). The gland consists of a reservoir sac lined with an epithelium of secretory cells. The reservoir connects directly with the lumen of the arolium (Fig. 4), and most likely this gland produces secretions for adhesion. The arolium is believed to serve as an adhesive organ to surfaces too hard or too smooth for

the claws to grasp (Snodgrass, 1956). This "arolium gland" has been found in all 6 legs of *Amblyopone* workers and resembles closely the tarsal glands Billen (1986) described in the wasp *Polistes annularis*.

The second type of tarsal gland found in *A. australis*, consists of a massive glandular cell cluster which is especially well developed in the elongated fifth tarsal segment of the hind legs (Fig. 5). This structure appears to be associated with a dense pattern of pore capillaries penetrating the cuticle of the ventral fifth tarsomere (Fig. 6). Such pore capillaries are absent in the dorsal part of the cuticle. The gland can also be found in the fifth tarsomere of the middle leg, where it is, however, poorly developed. It appears to be absent altogether in the front leg (Fig. 3).

DISCUSSION

A surprisingly large number of exocrine structures have been identified as sources for trail pheromones in ants (see reviews in Hölldobler 1984; Attygalle and Morgan 1986; Hölldobler and Wilson, 1990). In almost all species studied, trail pheromones originate from organs located in the gaster of the ants. The myrmicine genus *Crematogaster* is an exception, however, as the workers lay trails with secretions from a special tibial gland (Fletcher and Brand, 1968; Leuthold, 1968). For more detailed morphological descriptions of this gland see Pasteels et al. (1970) and Billen (1984).

In the present paper we report the presence of tarsal glands in the hind legs of *Amblyopone australis* which, considering the complementary behavioral observations (Hölldobler and Markl, unpublished), are suspected as a source for trail pheromones. It is possible that footprint marking is a more general phenomenon in ants than previously thought. In fact, several ant species are known to employ chemical "sign posts" for home range marking and orientation. In addition some species have recently been demonstrated to deposit colony specific and even individual specific chemical orientation cues (see Jessen and Maschwitz, 1986; for review see Hölldobler and Wilson, 1990). In most of these cases we do not yet know the exact anatomical source of these specific marking pheromones. Our discovery of tarsal "footprint glands" suggests new possibilities for the investigation of chemical marking signals in ants.

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